Forensic Science International: Genetics 10 (2014) 33–39

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Short Communication

Internal validation of the GlobalFiler™ Express PCR Amplification Kit for the direct amplification of reference DNA samples on a high-throughput automated workflow

Shahida Flores [a,*], Jie Sun [a], Jonathan King [a], Bruce Budowle [a,b]

[a] Institute of Applied Genetics, Department of Forensics and Investigative Genetics, University of North Texas Health Science Center, Fort Worth, TX 76107, USA
[b] Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia

ARTICLE INFO

Article history:
Received 5 December 2013
Received in revised form 14 January 2014
Accepted 18 January 2014

Keywords:
Short tandem repeats
Forensic STR loci
Y-indel
Direct amplification
Reference DNA samples
GlobalFiler Express™

ABSTRACT

The GlobalFiler™ Express PCR Amplification Kit uses 6-dye fluorescent chemistry to enable multiplexing of 21 autosomal STRs, 1 Y-STR, 1 Y-indel and the sex-determining marker amelogenin. The kit is specifically designed for processing reference DNA samples in a high throughput manner. Validation studies were conducted to assess the performance and define the limitations of this direct amplification kit for typing blood and buccal reference DNA samples on various punchable collection media. Studies included thermal cycling sensitivity, reproducibility, precision, sensitivity of detection, minimum detection threshold, system contamination, stochastic threshold and concordance. Results showed that optimal amplification and injection parameters for a 1.2 mm punch from blood and buccal samples were 27 and 28 cycles, respectively, combined with a 12 s injection on an ABI 3500xL Genetic Analyzer. Minimum detection thresholds were set at 100 and 120 RFUs for 27 and 28 cycles, respectively, and it was suggested that data from positive amplification controls provided a better threshold representation. Stochastic thresholds were set at 250 and 400 RFUs for 27 and 28 cycles, respectively, as stochastic effects increased with cycle number. The minimum amount of input DNA resulting in a full profile was 0.5 ng, however, the optimum range determined was 2.5–10 ng. Profile quality from the GlobalFiler™ Express Kit and the previously validated AmpFlSTR® Identifiler® Direct Kit was comparable. The validation data support that reliable DNA typing results from reference DNA samples can be obtained using the GlobalFiler™ Express PCR Amplification Kit.

1. Introduction

For nearly two decades, short tandem repeat markers (STRs) have been the mainstay for human identification testing in the forensic community [1]. Recently, with the demand for laboratories to process larger numbers of reference DNA samples, commercial manufacturers have released several direct amplification kits as well as supplementary robotic instrumentation to maximize throughput [2–5]. Direct amplification eliminates the traditional extraction and quantification steps thereby reducing the time and labor involved in processing reference DNA samples. In addition, the European Network of Forensic Science Institutes (ENFSI), the European DNA Profiling Group (EDNAP) and the Federal Bureau of Investigation (FBI) have outlined the need for an increased number of STR loci than was required previously [6,7]. With this requirement of expansion of core database STR loci, the GlobalFiler™ Express PCR Amplification Kit (Life Technologies, Carlsbad, CA) was developed. The kit uses 6-dye fluorescent chemistry to enable typing of 21 autosomal STRs, a Y-STR, a Y chromosome indel, and the sex-determining marker amelogenin. Combined with direct amplification chemistry and robotics, blood and buccal reference DNA samples on punchable media can be typed in a highly efficient automated workflow.

This paper describes validation studies conducted using the GlobalFiler™ Express PCR Amplification Kit, based on internal validation criteria in accordance with the Scientific Working Group on DNA Analysis Methods (SWGDAM) Quality Assurance Standards for DNA Databasing Laboratories [8]. The findings support that the kit produces reliable DNA typing results from blood and buccal reference DNA samples that would be of sufficient quality for uploading into DNA databases.

2. Materials and methods

2.1. Preparation of samples

Ten individuals donated blood and buccal samples for this study with the University of North Texas Health Science Center

* Corresponding author. Tel.: +1 817 735 2292; fax: +1 817 735 5016.
E-mail address: shahida.flores@unthsc.edu (S. Flores).

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http://dx.doi.org/10.1016/j.fsigen.2014.01.005

Institutional Review Board approval. Blood samples were obtained by finger-prick using a BD Microtainer™ Contact-Activated Lancet (BD, Franklin Lakes, NJ) and spotted directly on to Fitzco FP705™ Collection Cards (Fitzco, Inc., Spring Park, MN) as well as Whatman™ FTA® Classic Cards (GE Healthcare Life Sciences, Piscataway, NJ). Buccal samples were obtained with Bode Buccal DNA Collectors™ (Bode Technology, Lorton, VA) as well as Whatman™ EasiCollect™ Devices (GE Healthcare Life Sciences). Samples were maintained at ambient temperature and allowed to dry approximately 1–2 weeks before testing. Two individuals also donated liquid blood by venipuncture into a Vacutainer® tube with EDTA (BD) and saliva into a 15 mL conical tube for the sensitivity study.

2.2. Amplification

Unless otherwise noted, the manufacturer's recommendations from the GlobalFiler™ Express User Guide (Life Technologies) for processing blood and buccal samples on treated (i.e., FTA® paper) and untreated (i.e., non-FTA® paper) media were followed [9]. For each treated sample, a total reaction volume of 15 μL consisting of 6 μL of GlobalFiler™ Express Master Mix, 6 μL of GlobalFiler™ Express Primer Set and 3 μL of low TE buffer (10 mL Tris–HCl, pH 7.5; 200 μL 0.5 M EDTA, pH 8.0; 989.8 mL H_2O) was added to each well on a MicroAmp® Optical 96-Well Reaction Plate (Life Technologies) before a 1.2 mm disk of sample embedded in paper substrate was punched into the well. For each untreated sample, 3 μL of Prep-n-Go™ lysis buffer (Life Technologies) were added before a 1.2 mm disk of sample embedded in paper substrate was punched into the well. A reaction volume of 12 μL consisting of 6 μL of GlobalFiler™ Express Master Mix and 6 μL of GlobalFiler™ Express Primer Set was added for a total reaction volume of 15 μL. Sample punching was performed using a BSD600 Duet Series II Semi-Automated Punching System (Luminex Corporation, Austin, TX). Three cleaning punches from a blank substrate (i.e., no DNA) were made between each sample to reduce carryover. Liquid transfer was performed using a Hamilton MICROLAB® STARlet Liquid Handling Platform (Hamilton Company, Reno, NV). All amplifications were performed on an ABI GeneAmp® PCR System 9700 thermal cycler with a gold-plated sample block (Life Technologies). The ramping mode was set to 'Max' and the thermal cycling conditions were as follows: initial incubation at 95 °C for 1 min followed by 27 cycles for blood samples or 28 cycles for buccal samples (determined during the cycle sensitivity study) consisting of denaturation at 94 °C for 3 s and annealing/extension at 60 °C for 30 s. A final extension was set at 60 °C for 8 min followed by a final hold at 4 °C. Total thermal cycling time was approximately 40 min.

2.3. Capillary electrophoresis and data analysis

Samples were prepared by adding 1 μL of the PCR product or allelic ladder to the corresponding well on the CE plate which contained 10 μL of the formamide and size standard solution (9.5 μL of HiDi™ Formamide and 0.5 μL of GeneScan LIZ600 Size Standard v2.0, Life Technologies). Liquid transfer was performed using a Tecan Freedom EVO® 75 Liquid Handling Platform (Tecan US Inc., Morrisville, NC). After plate preparation, samples were denatured at 95 °C for 3 min and then placed on ice for 3 min before being loaded for capillary electrophoresis. PCR products were separated and detected using POP-4™ polymer and an ABI 3500xL Genetic Analyzer (Life Technologies). Before the run, a spectral calibration using the DS-36 Matrix Standard (J6 Dye Set) was performed for 6-dye chemistry. Samples were injected for 12 s (determined during the cycle sensitivity study) at 13 kV with a run temperature of 60 °C and a run time of 1550 s. Data analysis was performed using GeneMapper ID-X (GMID-X) Software v1.2 (Life Technologies) and an in-house Excel™ workbook (PHASTR: peak height analysis for short tandem repeats) to analyze peak height data [10].

2.4. Cycle sensitivity study

A cycle sensitivity study was conducted to determine the appropriate PCR cycle number for each sample type and collection medium. Ten samples of each of the four sample-media combinations were used to evaluate cycle sensitivity. Blood samples were amplified at 25, 26 and 27 cycles; buccal samples were amplified at 26, 27, and 28 cycles following the manufacturer's recommendations. Additionally, the amount of the positive control DNA 007 (2 ng/μL) was adjusted based on the manufacturer's recommended input volume for each cycle number: 3 μL for 25 and 26 cycles, 2 μL for 27 cycles and 1 μL for 28 cycles. The same ABI GeneAmp® PCR System 9700 thermal cycler was used to amplify each plate. Two injection times, 12 s and 24 s, were evaluated.

2.5. Reproducibility and precision study

Four sample-media combinations using five different individuals as DNA sources were amplified in triplicate using the manufacturer's recommendations and the appropriate cycle number to evaluate the reproducibility of the peak heights, peak balance and allele calls. Replicate sample punches were made within a few millimeters of each other in an effort to reduce intra-sample variation. Additionally, allelic ladders and positive controls were used to determine the precision of the size calling (in base pairs) and allele designation. The plate was injected a total of five times and the average and standard deviation of the base pair calling for each fragment was calculated.

2.6. Minimum threshold calculation and contamination assessment

Data from 64 negative amplification controls were pooled and used to assess baseline noise and calculate a minimum detection threshold for the GlobalFiler™ Express PCR Amplification Kit at 27 cycles for blood samples and 28 cycles for buccal samples. In the GMID-X analysis method, the peak amplitude threshold was adjusted to 1 relative fluorescent unit (RFU) to capture all data points and the analysis range was modified to correspond to the expected range of fragment sizes (75–475 bp). Peak heights attributed to spikes were removed from the data set and the average RFU values as well as the standard deviation values of the remaining peak heights for each dye channel were calculated. In addition, data from 8 positive amplification controls for 27 cycles (4 ng total input DNA) and 8 positive amplification controls for 28 cycles (2 ng total input DNA) were evaluated using the same analysis method as the negative amplification controls with the exception that peaks from alleles, forward stutter, reverse stutter, pull-up and, in some cases, other PCR products (i.e., −2 bp PCR products), were also removed before the average and standard deviation of the remaining peak heights were calculated.

The possibility of contamination was evaluated by using zebra and checkerboard patterns for plate layouts with staggered samples and blank substrates. These layouts assessed potential carryover contamination as the instruments moved vertically and/or horizontally during punching (BSD600 Duet Semi-Automated Punching System), dispensing (Hamilton MICROLAB STARlet Liquid Handling Platform, Tecan Freedom EVO 75 Liquid Handling Platform) and injecting (ABI 3500xL Genetic Analyzer).

2.7. Sensitivity and stochastic study

The sensitivity of detection of the GlobalFiler™ Express PCR Amplification Kit was assessed to estimate the range of total input DNA that would generate a full profile that meets the threshold criteria. Stochastic effects were evaluated using heterozygous peak height ratios from these data sets to help determine the stochastic threshold. Liquid blood and saliva from two individuals were diluted serially to yield 5 additional concentrations. Dilutions for the liquid blood were 1:2, 1:10, 1:50, 1:100 and 1:250. Dilutions for the saliva were 1:2, 1:5, 1:10, 1:25 and 1:50. One microliter of each concentration was spotted on a blank 1.2 mm disk from treated and untreated media and allowed to dry before further processing. Samples were amplified using manufacturer's recommendations for the sample-media combination. Additionally, extracted DNA was diluted serially to a range of total input DNA amounts (15 ng, 10 ng, 5 ng, 2.5 ng, 1 ng, 0.5 ng, 0.25 ng, 0.1 ng and 0.05 ng) and amplified at 27 and 28 cycles with the blood and buccal samples respectively. All samples were processed and amplified in duplicate.

2.8. Concordance check

A small concordance check was performed by typing 50 samples varying in ethnicity using the GlobalFiler™ Express PCR Amplification Kit and comparing the typing results with those obtained with AmpFlSTR® NGM SElect™ and AmpFlSTR® Identifiler® Direct PCR Amplification Kits (Life Technologies).

3. Results and discussion

3.1. Cycle sensitivity study

Data analyses for each sample-media combination were generated using the PHASTR workbook to globally evaluate the profiles at each variable. A representative worksheet can be seen in Fig. 1. Ideally, the optimal cycle number should result in a maximum number of alleles from each sample displaying heterozygous peak heights between 3000 and 12,000 RFUs, there should be no occurrence of allelic dropout and minimal occurrence of off-scale peaks [9]. However, peak heights falling moderately outside this range are still acceptable.

Both blood and buccal samples produced acceptable profiles at all tested conditions with a few exceptions. Only one treated buccal sample produced dropout at almost all conditions most likely due to insufficient DNA. Given the results, amplification using a higher cycle number and electrophoresis using a lower injection time were selected for each sample-media combination. The selected conditions were 27 cycles for blood samples and 28 cycles for buccal samples combined with a 12 s injection time. These conditions yielded the greatest number of successful results and allowed greater flexibility for reanalysis for those few samples that did not yield a complete profile or were overloaded (e.g., a longer or shorter re-injection time, respectively). Since the injection time was reduced from the manufacturer's recommendations, the amount of positive control was increased by 1 μL to obtain a more acceptable profile. Under these conditions, 65% (treated blood), 77% (treated buccal), 43% (untreated blood) and 61% (untreated buccal) of all peaks were between 3000 and 12,000 RFUs. Outside of this suggested range, more than 92% of all peaks were between 500 and 20,000 RFUs which were still acceptable. Most peaks up to 20,000 RFUs did not display associated pull-up but above this value pull-up was almost always apparent. Additionally, there were only a few occurrences where the peak height ratio (PHR) fell below 0.7:4 (2.4%) for treated buccal, 12 (7.2%) for untreated blood and 9 (5.5%) for untreated buccal.

3.2. Reproducibility and precision study

All samples in the reproducibility study yielded full profiles with the exception of one untreated buccal sample replicate

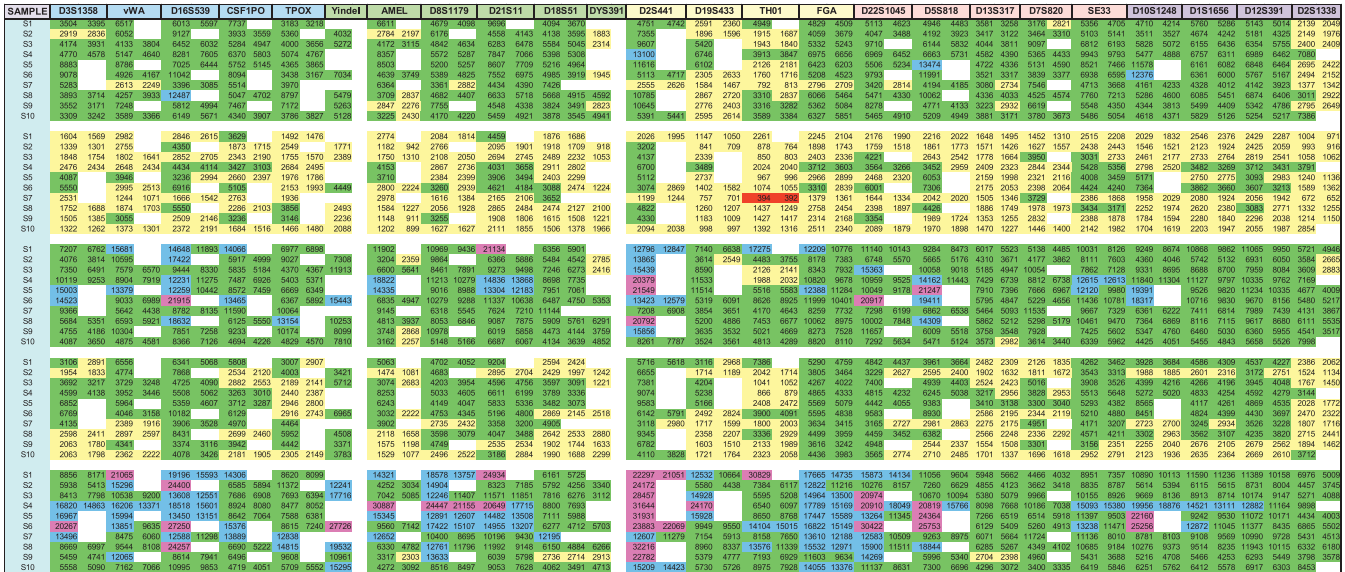

Fig. 1. Peak heights for 10 treated blood samples amplified at 25, 26 and 27 cycles and injected at 12 and 24 s. The following peak height RFU ranges were highlighted: <500 RFUs (red), 500–2999 RFUs (yellow), 3000–12,000 RFUs (green), 12,000–20,000 RFUs (blue) and >20,000 RFUs (pink). While all conditions returned full profiles, the highest cycle number and lowest injection time tested was chosen. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

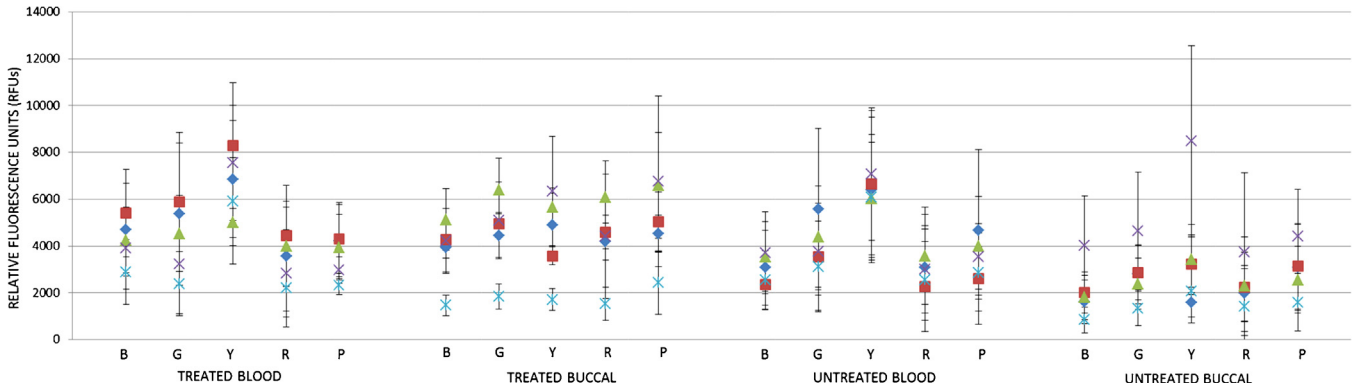

Fig. 2. Average peak heights and standard deviation for each dye channel. Five donors (amplified in triplicate) are represented by the symbols.

displaying dropout at the D7S820 locus. All allele calls were concordant across the replicates.

The average peak heights as well as standard deviations across dye channels for the four sample types are displayed in Fig. 2. Generally, treated samples yielded higher peak heights and lower standard deviations than untreated samples. Unlike traditionally extracted and quantified samples where total input DNA can be measured, the amount of DNA obtained from 1.2 mm punches in direct amplification can be highly variable based on sample distribution. Therefore, increased intra-sample peak height variation across replicates was expected.

Overall, heterozygous balance was within acceptable limits with most markers displaying peak height ratios above 0.7. A total of 252 heterozygous loci across individuals and replicates were examined. Untreated samples were found to have higher occurrences of imbalance than treated samples: 23 for untreated blood, 20 for untreated buccal, 5 for treated blood and 2 for treated buccal. Markers that displayed imbalance more so than others were the SE33 and D2S1338 loci; both consist of the largest sized amplicons in the red and purple dye channels, respectively.

Intracolor balance, the uniformity of peak heights across loci in a dye channel, was examined by taking the average peak heights for each locus in a dye channel and then comparing minimum peak height to maximum peak height as a percentage (Table 1) [11]. Generally, RFU values within a dye channel decreased as amplicon size increased. Most noted was the peak height decrease at the DYS391 marker (largest amplicon in green dye channel) compared to the Y-indel marker (smallest amplicon in green dye channel). Also, some loci such as CSF1PO, D13S317, D7S820 and D18S51 tended to display lower peak heights across most profiles and sample-media combinations when compared to the other loci. Overall, treated samples, especially buccal, had better intracolor balance than untreated samples. An example profile can be seen in Fig. 3. While intracolor balance ratios are lower compared to results generally observed in forensic casework kits (data not shown), it is not as important for direct amplification kits; the profiles are single source and, generally, sufficient DNA is available for analysis.

Table 1
Comparison of intracolor balance for sample types expressed as mean (%) ± SD.

Dye channel	Treated blood	Untreated blood	Treated buccal	Untreated buccal
Blue	33.5 ± 7.6	28.6 ± 3.3	52.5 ± 7.9	25.0 ± 5.9
Green	27.8 ± 13.3	18.0 ± 6.2	59.8 ± 12.6	27.4 ± 5.7
Yellow	45.1 ± 3.0	38.2 ± 5.2	58.2 ± 19.0	37.3 ± 6.9
Red	23.9 ± 8.2	15.1 ± 5.0	49.7 ± 20.1	15.1 ± 5.6
Purple	49.9 ± 17.5	23.2 ± 12.0	51.0 ± 13.2	26.1 ± 10.0

In the precision study, the sizing results revealed that all positive control alleles fell within the ±0.5 bp range of the corresponding alleles in the allelic ladder. Furthermore, allelic ladder sizing precision showed 0.13 bp as the largest standard deviation of allele sizes obtained across all injections. This largest value was obtained twice in the TPOX marker at allele 12 and 14 in different capillaries. Target value for standard deviation was 0.15 bp or less. Based on these values, sizing accuracy and precision met performance criteria. The system was fully capable of correct base pair sizing down to 1 bp resolution.

3.3. Minimum threshold calculation and contamination assessment

Minimum threshold (i.e., analytical threshold or peak amplitude threshold) was defined as the set RFU value that separates, with a high degree of confidence, true DNA peak signal from background noise [12]. There are several methods for calculating the minimum threshold value [13]. The main method used in this study, was similar to that originally proposed by Kaiser [14] to determine an analytical threshold based on reliable separation of signal from noise using a quantitation limit.

For both negative and positive amplifications at 27 and 28 cycles, the limit of quantification (LOQ) was determined for each dye channel by using ten times the standard deviation above the average background RFU value (Table 2). The peak amplitude threshold in the GMID-X analysis method was set to the LOQ of the 'noisiest' dye channel (the green dye channel in all cases); however, it can also be set to be dye-dependent.

For positive amplifications, the 27 cycle amplification consisted of 4 ng total input DNA while the 28 cycle amplification consisted of 2 ng total input DNA. The LOQ value was higher for the 28 cycles indicating that background noise increased with cycle number irrespective of DNA amount. However, DNA amount does play a large role as samples with higher DNA amounts generated more PCR products and pull-up. Also, markers SE33 and D1S1656 consistently produced PCR products 2 bp less in size than the true peak in addition to regular forward and reverse stutter products. This is most likely due to the repeat motif of these two markers and has been seen previously [11]. The maximum background RFU values from the positive amplification control data tended to exceed the calculated minimum threshold from the negative amplification control data indicating the presence of template DNA can influence the presence of peaks and peak heights in non-allele product areas in each dye channel (data not shown). These data support that consideration should be given to using positive control samples instead of negative control samples for establishing noise levels. The greater noise in positive samples would seem to reflect better the noise levels in database samples (as well as forensic samples) than would negative controls. With this

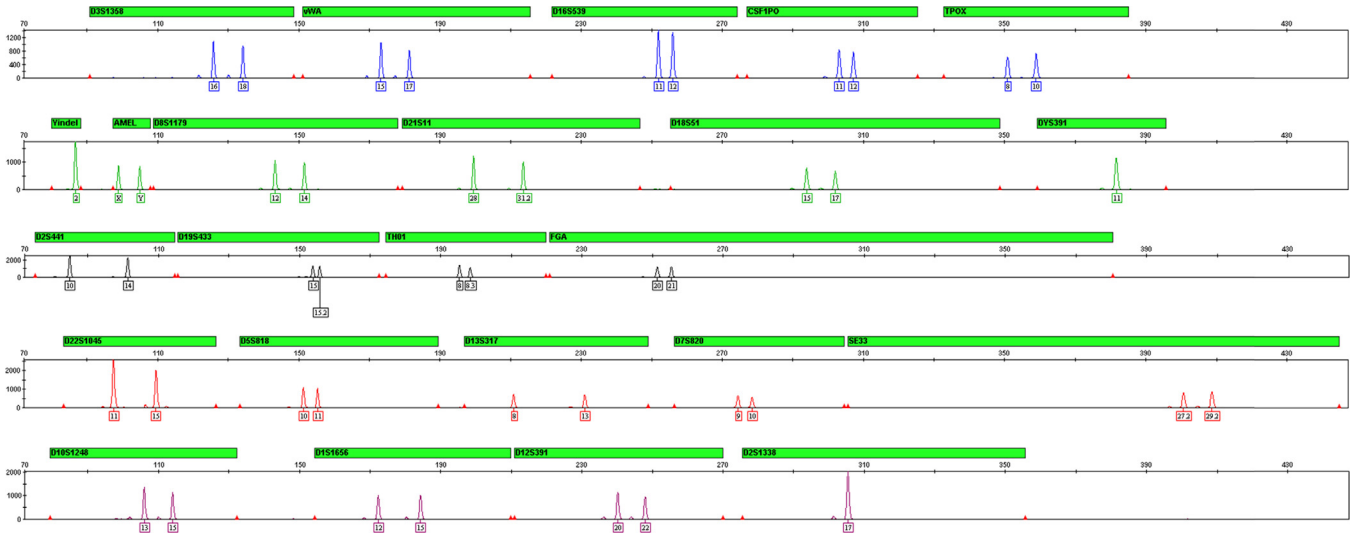

Fig. 3. Representative electropherogram of a male buccal sample on FTA® paper (treated buccal) amplified with the GlobalFiler™ Express PCR Amplification Kit for 28 cycles.

consideration, the more conservative values of 100 RFUs (27 cycles) and 120 RFUs (28 cycles) were chosen as the minimum thresholds. In addition, a global cut-off filter of 0.2 was employed for analyzing reference DNA samples.

During the contamination assessment, 11 of 64 negative amplification samples showed low level contamination below 100 RFUs. Most peaks were attributed to alleles from the sample that was punched immediately prior to the three clean punches and the blank substrate in each case. This contamination most likely originated from the punching system. During punching, a total of 3 cleaning strikes were performed between every sample which was the maximum number of cleaning strikes allowed by the manufacturer. While no contamination was detected in any of the reference DNA samples, this low level contamination in some of the blank substrate punches can be avoided by increasing the number of cleaning strikes between samples. Internal validation by each laboratory should take into account the number of cleaning strikes to determine the best fit.

3.4. Sensitivity and stochastic study

The sensitivity study examined the effect that various amounts of input template DNA had on peak height and heterozygote balance. Stochastic effects due to low template DNA can cause severe imbalance leading to dropout and it is important to characterize the level at which this is most likely to occur. Stochastic threshold (i.e., homozygous threshold) was defined as the set RFU value above which there is a high degree of confidence that if only one peak is observed, that peak is a true homozygote. If only one peak is observed below the stochastic threshold, but above the minimum threshold, there is a possibility that drop-out of a sister allele may have occurred. If the stochastic threshold is set too low, the risk of false homozygotes increases whereas if it is set too high, true homozygotes may be inconclusive and fewer full profiles may be generated [15].

With extracted DNA, the ideal range of total input DNA was 2.5–10 ng for both cycles (Table 3). In this range, there were no heterozygous peak height ratios under 0.7, minimum peak height values were 755 RFUs (27 cycles) and 1681 RFUs (28 cycles) and although a few peaks exceeded 20,000 RFUs, no saturation was observed. While both cycles were able to generate full profiles lower than this range (down to 0.5 ng), imbalance between peak heights increased as the template DNA decreased. More stochastic effects were seen with lower template DNA (0.1 and 0.05 ng) in the 28 cycle amplification than in the 27 cycle amplification.

With the dilution series for blood and saliva samples, the general trend was a decrease in peak height (RFUs) with each dilution with optimal recovery around 1:10 for blood samples and 1:5 for saliva samples. One microliter of undiluted sample may have overestimated the amount of DNA in a punch since inhibition effects were somewhat noticeable in the undiluted and 1:2 samples. Previous studies have estimated the DNA yield can vary depending on the biological source with liquid blood around 20–40 ng/μL and saliva around 4–30 ng/μL [16,17].

Peaks from heterozygotes with heights above the minimum threshold and sister alleles below the minimum threshold were selected for calculating the stochastic threshold for 27 cycles ($N = 69$) and 28 cycles ($N = 36$). The mean peak height plus three standard deviations was used to establish the stochastic threshold for each cycle number [18]. Based on these data sets, the suggested stochastic threshold is 250 RFUs for 27 cycles and 400 RFUs for 28 cycles. There were no false homozygotes for any of the samples using a minimum threshold of 100 RFUs (blood) and 120 RFUs (buccal) and a stochastic threshold of 250 RFUs (blood) and 400 RFUs (buccal). It is recommended that selection of thresholds be supported by internal validation studies performed by each laboratory.

Table 2
Average (AVE), standard deviation (SD) and limit of quantification (LOQ) in RFUs determined for each dye channel for negative and positive amplification controls. Noisiest dye channel in bold.

Sample	Cycle	Calc.	B	G	Y	R	P
Neg.	27	AVE	6	10	5	8	9
		SD	3	4	3	4	4
		LOQ	36	**50**	35	48	49
Neg.	28	AVE	7	11	5	9	10
		SD	7	7	4	7	7
		LOQ	77	**81**	45	79	80
Pos. (4 ng)	27	AVE	8	13	6	11	12
		SD	6	8	4	8	7
		LOQ	68	**93**	46	91	82
Pos. (2 ng)	28	AVE	8	16	6	12	14
		SD	5	10	5	9	10
		LOQ	58	**116**	56	102	114

Table 3
Stochastic effects observed during sensitivity study for heterozygous (Het.) and homozygous (Hom.) peaks in terms of peak height ratio (PHR) and peak heights (in RFUs) with varying inputs of template DNA for 27 and 28 cycles.

Cycle number	Template DNA (ng)	# of PHR under 0.7	Average PHR	Lowest PHR	Het. pair with lowest peak (RFUs)	Het. pair with highest peak (RFUs)	Lowest Hom. peak (RFUs)	Highest Hom. peak (RFUs)
27	0.05	28	0.52	0.14	11, 81	44, 163	36	126
	0.1	16	0.68	0.24	29, 123	117, 260	60	223
	0.25	11	0.75	0.41	57, 137	426, 446	104	470
	0.5	4	0.84	0.55	186, 263	836, 1172	340	1365
	1	2	0.86	0.62	492, 530	2127, 2424	644	2488
	2.5	0	0.91	0.73	784, 904	4947, 5002	1944	6954
	5	0	0.92	0.74	755, 844	5823, 6797	1164	10,558
	10	0	0.90	0.75	4279, 4607	13,304, 1413	5116	18,790
	15	0	0.90	0.79	3839, 4550	14,316, 14,806	4317	20,946
28	0.05	30	0.48	0.02	9, 382	84, 391	43	461
	0.1	20	0.65	0.15	46, 303	339, 532	36	817
	0.25	14	0.74	0.48	98, 146	778, 1019	226	1024
	0.5	0	0.85	0.70	396, 523	1664, 2291	568	2829
	1	3	0.87	0.59	1009, 1020	3888, 4288	1120	5855
	2.5	0	0.91	0.78	1681, 2001	10,892, 11,840	2598	14,959
	5	0	0.91	0.75	4606, 4919	13,805, 14,509	4986	23,066
	10	0	0.90	0.75	5049, 6718	17,901, 19,009	5505	26,503
	15	0	0.88	0.71	6327, 7838	18,769, 21,152	4823	31,721

3.5. Concordance study

The primer sequences for shared loci across the three kits were maintained with the exception of a modified reverse primer for the TPOX locus and addition of several degenerate primers in the GlobalFiler™ Express Kit [9]. Thus, concordance (for allele designations) was expected and supported by the data. Overall profile quality and sensitivity using the internally validated methods for the two direct amplification kits, Identifiler® Direct and GlobalFiler™ Express, were comparable in terms of first pass success rate, peak height and heterozygous balance. Profile quality and sensitivity were not compared to the casework kit, NGM SElect™, since sampling methodology and kit chemistry are different [9,11]. While not an indication of discordance, the Identifiler® Direct and NGM SElect™ kits resulted in more off-ladder (OL) allele designations than the GlobalFiler™ Express Kit. One recurrent example of this is the 10.3 allele in the D7S820 locus which has been previously documented [1]. The GlobalFiler™ Express bin sets incorporate more virtual bins that the other two kits thereby increasing automated allele designations and reducing the time required to manually evaluate and assign microvariant alleles. Additionally, the manual designation of microvariant alleles for outside marker range (OMR) calls varied based on the smallest or largest bin of the allelic ladder in the kit (e.g., >18.2 for Identifiler® Direct and NGM SElect™ and >19.2 for GlobalFiler™ Express at the D19S433 locus). The microvariant allele was the same distance apart (in bp) from its sister allele for all three kits. Although this is not an example of discordance, it may be important to consider when comparing profiles generated using different kits.

4. Conclusion

These studies demonstrate that the GlobalFiler™ Express PCR Amplification Kit produces reliable, robust and reproducible results when amplifying blood and buccal reference DNA samples on punchable media. Studies showed that the optimal amplification parameters for blood and buccal samples were 27 and 28 cycles, respectively, with an optimal input DNA range of 2.5–10 ng. Thresholds were based on amplification cycle number with the minimum threshold and stochastic threshold set at 100 RFUs and 250 RFUs, respectively, for 27 cycles and 120 RFUs and 400 RFUs, respectively, for 28 cycles.

Additionally, the short amplification time of this kit (~40 min) combined with various robotic systems, such as the BSD600-Duet Punching System and the 3500xL Genetic Analyzer, make it practical for a high-throughput automated workflow that generates good quality profiles in dramatically less processing time than traditional methods.

Acknowledgement

The authors would like to thank Life Technologies for its contributions to this study.

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